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TITLE: A PSCA Promoter Based Avian Retroviral Transgene Model of Normal and Malignant Prostate

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INTRODUCTION

The molecular and cellular origins of prostate cancer are poorly understood. Recent evidence from our laboratory suggests that prostate cancer may arise from a basal/luminal precursor cell marked by cell surface expression of PSCA. The evidence supporting this hypothesis is that (1) PSCA marks an intermediate cell population that co-expresses basal and luminal cell cytokeratins (2) this cell population does not express p63 and is androgen receptor positive, all hallmarks of prostate cancer, and (3) PSCA is highly expressed in HGPIN and prostate cancer and in all animal models of prostate cancer. To test this hypothesis and to develop new models of prostate, we propose to determine whether delivery of oncogenes specifically to the PSCA positive cells of mouse prostate is sufficient to cause cancer. To accomplish this, we will develop a transgenic mouse model in which the retroviral receptor gene *tva* is expressed in the prostate under control of the PSCA promoter. Virus containing one or more oncogenes will be delivered to the prostate and the resulting phenotype characterized.

PROGRESS REPORT

Specific Aim 1. Establishment of a PSCA-*tva* transgenic line and characterization of the TVA positive cell population. (months 1-18).

This Aim has been completed.

Task 1-2. Multiple PSCA-*tva* transgenic mice were established and TVA expression was confirmed in the prostate at the RNA and protein levels. Two transgenic lines were maintained and expanded for *in vivo* orthotopic experiments. Orthotopic delivery of virus was optimized and standardized, first using the marker gene GFP, and subsequently using luciferase. The latter marker enables us to monitor gene uptake into the prostate noninvasively using the CCD camera. (Figure 1) Orthotopic injection of virus into the dorsal lobe at 5 weeks of age resulted in reproducible uptake of virus and luciferase expression while control mice were negative.

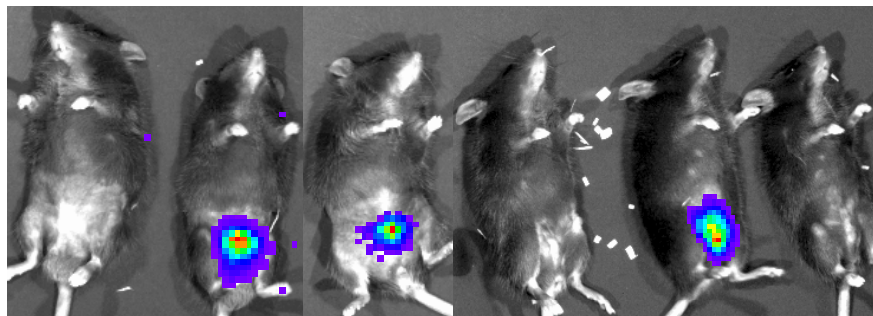


Figure 1: Expression of luciferase in the prostates of 3 PSCA-*tva* transgenic mice compared with 3 wild-type mice. All were injected with virus at 5 weeks of age orthotopically into the prostate and imaged with the CCD camera at 2 weeks later.

We performed additional experiments to determine if systemic administration of virus by intraperitoneal (IP) injection can improve prostatic uptake, since a limitation of the orthotopic approach is that virus uptake in the lateral and ventral lobes, which express high levels of *tva*, is suboptimal. IP injection of luciferase-expressing virus into five days old pups resulted in significant expression of luciferase in the pelvic and stomach regions. However luciferase expression decreased when the mice reached puberty and were not maintained further. In a parallel experiment, middle T virus were delivered via IP and mice sacrificed 3 months later, middle T

expression were clearly detected by in situ hybridization in the prostate and stomach. Based on this result, we combined both IP and orthotopic deliveries in our subsequent experiments.

Task 3-4. The PSCA-tva positive cells have furthermore been characterized to determine if they are equivalent to the human PSCA-positive prostate epithelial cells (i.e intermediate cells). We performed a recombination experiment, in which the PSCA-tva mouse prostate cells were mixed with mesenchymal cells isolated from the mouse urogenital sinus, and inoculated under the kidney capsule of immunodeficient mice. The resulting tissue, harvested at 8 weeks later, reconstituted prostate gland-like structures. When we looked for TVA expression by immunohistochemistry in the glands, higher signal was observed in what appeared to be developing gland while cells lining the mature glands had lower to negligible level of expression (Figure 2). This is consistent with the hypothesis that PSCA-tva is a marker of an intermediate precursor cell in the developing prostate. We proceeded to Aim 2 to determine if this cell type is transformable in this model.

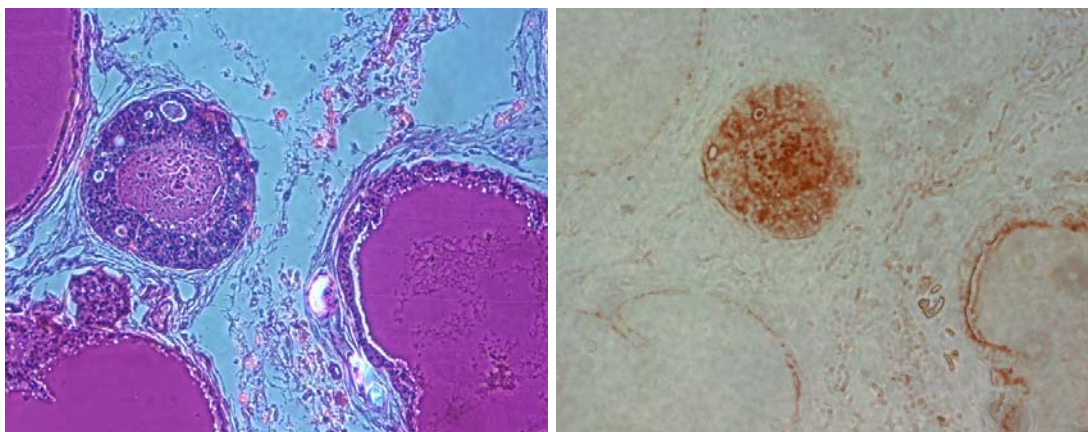


Figure 2: TVA expression in recombinant tissue from PSCA-tva mouse. H/E staining and immunohistochemistry of reconstituted prostate gland-like structure (x 400).

Specific Aim 2. Induction of cancer using the oncogene polyoma virus middle T antigen (months 12-36)

Task 1-3. Middle T antigen virus were delivered into 10 transgenic prostates and mice were then harvested at 6 months of age. We unexpectedly found that 50 % of mice developed large hemangiomas (Figure 3). Parallel experiments with middle T injection into bladder resulted in the same—large hemangiomas. It turns out that endothelial cells are exquisitely sensitive to transformation by middle T, which leads to hemangioma formation. Although there is no detectable TVA expression whatsoever in endothelium, it seems likely that the inflammation or trauma of orthotopic infection or very low-level expression of PSCA-tva by prostatic endothelium resulted in hemangioma formation. We performed *in situ* hybridization on these tissues, and found middle T mRNA expression also in the apparently normal prostate glands adjacent to the hemangioma (Figure 4). We also examined mice harvested at 10 months post middle T injection and found similar results, that hemangioma was present in some mice, but the epithelial glands were unaffected. This result demonstrated that while middle T can affect endothelial cells dramatically, its expression alone appeared insufficient to cause abnormality in the prostate epithelium. However, it is evident that genes delivered by this system can be stably expressed in long term (6 months post injection), even though the co-injected luciferase signal was no longer observed.

To overcome the above mentioned problems, we moved on to other oncogenes and combinations of oncogenes, which would be unlikely to cause an effect secondary to any low level background. Mice were inoculated with either myc virus (n=15), or activated Akt (n=10), or both (n=8). To date, the myc group was harvested at one year post injection and examined histologically. Mild hyperplasia was observed in 2 out of 15

mice, suggesting that just like middle T, expressing a single oncogene in a subset of cells in the prostate may not be enough to cause transformation (Figure 5). The Akt group and the combination group are being collected and examined for histological changes.

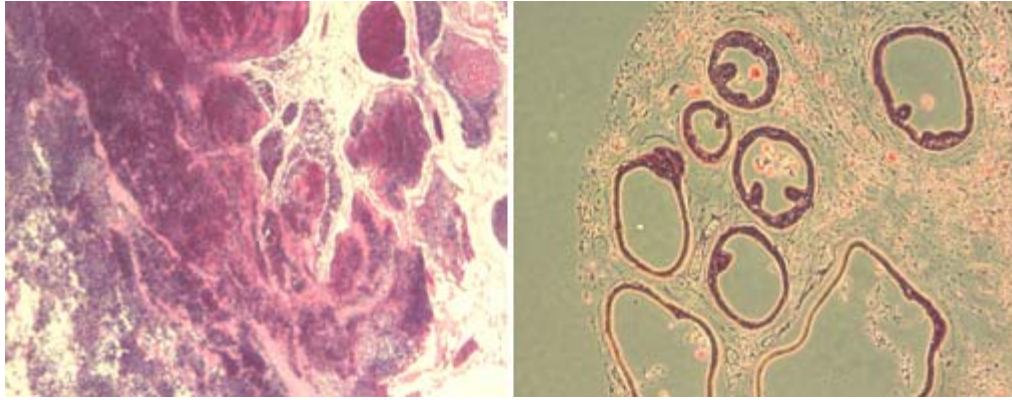


Figure 3: Histology of PSCA-tva mouse prostate injected with middle T antigen, sacrificed at 6 months. Large hemangioma was presented around and within the dorsal prostate (left panel), while remnants of normal prostate glands were also observed in adjacent area (right panel).

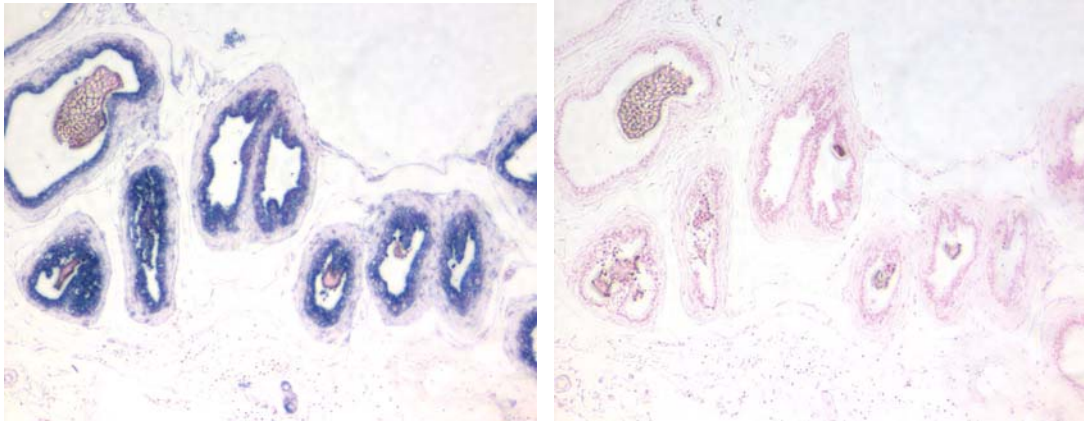


Figure 4: Expression of middle T antigen, detected by *in situ* hybridization, in the dorsal prostate adjacent to the hemangioma found in PSCA-tva mouse injected with middle T antigen. Antisense (left panel); sense control (right panel).

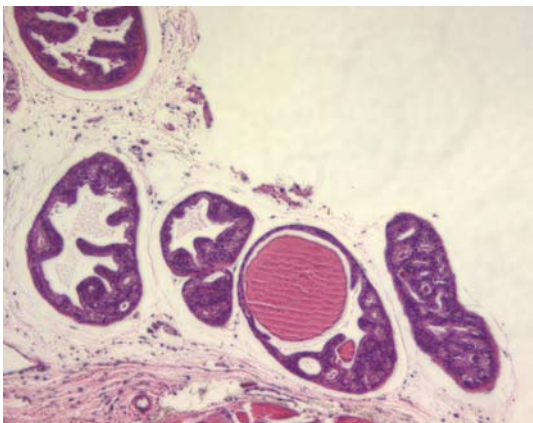


Figure 5: Mild hyperplasia in prostate gland of PSCA-tva mice injected with myc oncogene at one year post injection.

Also, as planned in our study, PSCA-tva mice were crossed with Nkx3.1 knockout. This knockout model was chosen over other more aggressive models (like PTEN knockout) because we want to see the effects of the introduced genes. To date, we have inoculated the bigenic mice (PSCA-tva / NKX3.1 knockout) with myc (n=15), akt (n=10), and both (n=8). These groups are monitored and will be harvested at 1 year post injection. Furthermore, we crossed PSCA-tva mice with PTEN flox mice and have started to infect the bigenic offspring with cre expressing virus to inactivate PTEN in tva-positive cells. We hope that these solutions will result in models that are more analogous to human prostate cancer. Of note however, while observing reports from other groups using this system in other organs, we have realized that the tva system works best for organs that are solid mass, such as the brain or liver. For our system, the efficiency of infection would be much lower since the mouse prostate is composed of a numbers glands, not a single solid mass, therefore it is difficult to infect every glands with one single injection.

KEY RESEARCH ACCOMPLISHMENTS OVER YEAR 2-3:

- Combination strategies for better gene delivery: both IP and orthotopic routes.
- Demonstration of long-term stable expression of delivered genes.
- Delivery of other potential oncogenes to PSCA-tva mice (myc and activated Akt).
- Observation that Myc caused mild hyperplasia.
- Generation of bigenic mice: PSCA-tva / NKX3.1 knockout, and PSCA-tva / PTEN flox
- Delivery of oncogenes to bigenic offspring.

REPORTABLE OUTCOMES

None

CONCLUSION: We have made significant progress over the past year. The major accomplishment has been the demonstration that in this system, virus can be inoculated orthotopically and the delivered gene expressed stably by the prostate in long term. In addition, we have proceeded to apply this model to screen various genes' capability, either introduced alone or in combination, to induce transformation in the prostate. We hope to collect and process all data from ongoing experiments in the next few months to provide a better understanding of how well the model can work.